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(54) Title: TUMOR HOMING MOLECULES, CONJUGATES DERIVED THEREFROM, AND METHODS OF USING SAME

(57) Abstract

The present invention provides tumor homing molecules, which selectively home to a tumor. The invention also provides methods of using a tumor homing molecule to target an agent such as a drug to a selected tumor or to identify the target molecule expressed by the tumor. The invention also provides methods of targeting a tumor containing angiogenic vasculature by contacting the tumor with a molecule that specifically binds an α_v-containing integrin. The invention further provides molecules that can selectively home to angiogenic vasculature. In addition, the invention provides a target molecule, which is specifically bound by a tumor homing molecule and is expressed by angiogenic vasculature. The invention also provides antibodies that bind to the target molecule and peptidomimetics that competitively inhibit binding of a ligand to the target molecule.

We claim:

1. A conjugate, comprising a tumor homing peptide linked to a moiety, said tumor homing peptide obtained by *in vivo* panning, comprising the steps of:

a) administering to a first subject having a tumor a library of diverse peptides;

b) collecting a sample of the tumor;

c) identifying a peptide that homes to said tumor;

d) collecting a sample of normal tissue corresponding to said tumor; and

e) determining that said peptide that homes to said tumor is not present in said normal tissue, thereby obtaining said tumor homing peptide,

provided said tumor homing peptide is not an antibody.

2. The conjugate of claim 1, wherein said peptide contains the amino acid sequence RGD.

3. The conjugate of claim 1, wherein said peptide contains the amino acid sequence NGR.

4. The conjugate of claim 1, wherein said peptide contains the amino acid sequence GSL.

SEQ ID NO: 5

6. The conjugate of claim 1, wherein said tumor homing peptide is CDCRGDCFC (SEQ ID NO: 1).

7. The conjugate of claim 1, wherein said tumor homing peptide is CNGRCVSGCAGRC (SEQ ID NO: 3) or CGSLVRC (SEQ ID NO: 5).

8. The conjugate of claim 1, wherein said moiety is a cytotoxic agent.

9. The conjugate of claim 1, wherein said moiety is a drug.

10. The conjugate of claim 9, wherein said drug is a cancer chemotherapeutic agent.

11. The conjugate of claim 10, wherein said cancer chemotherapeutic agent is doxorubicin.

12. The conjugate of claim 1, wherein said moiety is a detectable moiety.

13. The conjugate of claim 1, wherein said moiety is selected from the group consisting of a chambered microdevice, a liposome, a cell and a virus.

14. The conjugate of claim 1, wherein said moiety is a grafted polypeptide.

15. A conjugate, comprising a tumor homing molecule linked to a moiety, said tumor homing molecule obtained by *in vivo* panning, comprising the steps of:

a) administering to a first subject having a tumor a library of diverse molecules;

b) collecting a sample of the tumor;

c) identifying a molecule that homes to said tumor;

d) collecting a sample of normal tissue corresponding to said tumor; and

e) determining that said molecule that homes to said tumor is not present in said normal tissue, thereby obtaining said tumor homing molecule,

provided said tumor homing molecule is not an antibody.

16. The conjugate of claim 15, wherein said molecule is a nucleic acid molecule.

17. The conjugate of claim 15, wherein said molecule is a peptidomimetic.

18. A conjugate, comprising a tumor homing peptide containing the amino acid sequence RGD, said tumor homing peptide linked to a moiety.

19. The conjugate of claim 18, wherein said

20. A conjugate, comprising a tumor homing peptide containing the amino acid sequence NGR, said tumor homing peptide linked to a moiety.

21. The conjugate of claim 20, wherein said tumor homing peptide is NGRAHA (SEQ ID NO: 6).

22. The conjugate of claim 20, wherein said tumor homing peptide is CNGRC (SEQ ID NO: 8).

23. The conjugate of claim 20, wherein said tumor homing peptide is CNGRCVSGCAGRC (SEQ ID NO: 3).

24. A conjugate, comprising a tumor homing peptide containing the amino acid sequence GSL, said tumor homing peptide linked to a moiety.

25. The conjugate of claim 24, wherein said tumor homing peptide is CGSLVRC (SEQ ID NO: 5).

26. A conjugate, comprising a tumor homing peptide selected from the group consisting of NGRAHA (SEQ ID NO: 6) and CNGRC (SEQ ID NO: 8), said tumor homing peptide linked to a moiety.

27. A conjugate, comprising CDCRGDCFC (SEQ ID NO: 1) linked to a moiety.

28. A conjugate, comprising a tumor homing peptide selected from the group consisting of CNGRCVSGCAGRC (SEQ ID NO: 3) and CGSLVRC (SEQ ID NO: 5), said tumor homing peptide linked to a moiety.

29. A tumor homing peptide identified by *in vivo* panning, comprising the steps of:

a) administering to a first subject having a tumor a library of diverse peptides;

b) collecting a sample of the tumor;

c) identifying a peptide that homes to said tumor;

d) collecting a sample of normal tissue corresponding to said tumor; and

e) determining that said peptide that homes to said tumor is not present in said normal tissue, thereby identifying said peptide as a tumor homing peptide,

provided said peptide is not an antibody.

30. The tumor homing peptide of claim 29, wherein said sample of normal tissue corresponding to said tumor is collected from said first subject.

31. The tumor homing peptide of claim 29, wherein said sample of normal tissue corresponding to said tumor is collected from a second subject.

32. The tumor homing peptide of claim 29, wherein said tumor is a breast tumor.

33. The tumor homing peptide of claim 29, wherein said tumor is a melanoma.

wherein said tumor is a kaposi's sarcoma.

35. A tumor homing peptide selected from the group consisting of CNGRCVSGCAGRC (SEQ ID NO: 3) and CGSLVRC (SEQ ID NO: 5).

36. A tumor homing molecule identified by *in vivo* panning, comprising the steps of:

a) administering to a first subject having a tumor a library of diverse molecules;

b) collecting a sample of the tumor;

c) identifying a molecule that homes to said tumor;

d) collecting a sample of normal tissue corresponding to said tumor; and

e) determining that said molecule that homes to said tumor is not present in said normal tissue, thereby identifying said molecule as a tumor homing molecule,

provided said molecule is not an antibody.

37. A method of directing a moiety to a tumor, comprising contacting the tumor with the conjugate of claim 1.

38. The method of claim 37, wherein said contacting step is performed *in vitro*.

39. The method of claim 37, wherein said contacting step is performed *in vivo*.

40. A method of identifying the presence of a target molecule, which specifically binds a tumor homing molecule, wherein said tumor homing molecule is not an antibody, comprising contacting a sample of a tumor with the tumor homing molecule and detecting specific binding of said tumor homing molecule to a component of said sample, said binding identifying the presence of a target molecule.

41. A method of identifying a target molecule, which is expressed in a tumor tissue, comprising the steps of:

a) contacting a tumor tissue sample with a tumor homing molecule that specifically binds to said tumor tissue, wherein said tumor homing molecule is not an antibody;

b) identifying in said tumor tissue sample target molecules bound by said tumor homing molecule;

c) contacting a corresponding nontumor tissue sample with said tumor homing molecule;

d) identifying in said corresponding nontumor tissue sample target molecules bound by said tumor homing molecule; and

e) comparing said target molecules of said tumor tissue with said target molecules of said corresponding nontumor tissue, thereby identifying a target molecule expressed by said tumor tissue, wherein said target molecule

42. A method of obtaining a substantially isolated target molecule, which specifically binds a tumor homing molecule, comprising the step of substantially isolating the target molecule identified by the method of claim 41.

43. A substantially isolated target molecule, which specifically binds a tumor homing molecule, obtained by the method of claim 42, provided said target molecule is not an integrin.

44. A peptidomimetic, which competitively inhibits the binding of the target molecule of claim 43 to a naturally occurring ligand of the target molecule.

45. A molecule, which specifically binds to the target molecule of claim 43.

46. A molecule, which competitively inhibits the binding of the target molecule of claim 43 to the tumor homing molecule.

47. The molecule of claim 46, which is a peptide.

48. The peptide of claim 47, which contains the amino acid sequence NGR.

49. The peptide of claim 48, which has the amino acid sequence CNGRCVSGCAGRC (SEQ ID NO: 3).

50. The peptide of claim 47, which contains the amino acid sequence GSL.

51. The peptide of claim 50

52. A target molecule, which is expressed in tumor vasculature, wherein said target molecule binds CNGRC (SEQ ID NO: 8) with a higher affinity than said target molecule binds CDCRGDCFC (SEQ ID NO: 1).

53. An antibody that specifically binds the target molecule of claim 43.

54. The antibody of claim 53, which is a monoclonal antibody.

55. A method of directing a moiety *in vivo* to a tumor containing angiogenic vasculature, comprising contacting the tumor with the conjugate of claim 1.

56. The method of claim 55, wherein said conjugate comprises a peptide containing the amino acid sequence RGD.

57. The method of claim 55, wherein said conjugate comprises a peptide containing the amino acid sequence NGR.

58. The method of claim 55, wherein said conjugate comprises a peptide containing the amino acid sequence GSL.

59. The method of claim 55, wherein said conjugate comprises CDCRGDCFC (SEQ ID NO 1).

60. The method of claim 55, wherein said conjugate comprises NGRAHA (SEQ ID NO: 6) or CNGRC (SEQ ID NO: 8).

GSLVRG (SEQ ID NO:

62. The method of claim 55, wherein said conjugate comprises a moiety, which is a cytotoxic agent.

63. The method of claim 55, wherein said conjugate comprises a moiety, which is a drug.

64. The method of claim 63, wherein said drug is a cancer chemotherapeutic agent.

65. The method of claim 64, wherein said cancer chemotherapeutic agent is doxorubicin.

66. The method of claim 55, wherein said conjugate comprises a moiety, which is a detectable moiety.

67. The method of claim 55, wherein said conjugate comprises a moiety selected from the group consisting of a chambered microdevice, a liposome, a cell and a virus.

68. The method of claim 55, wherein said conjugate comprises a moiety, which is a grafted polypeptide.

69. A method of targeting *in vivo* a tumor containing angiogenic vasculature, comprising contacting the tumor with a molecule that selectively binds an α_v-containing integrin.

70. The method of claim 69, said molecule selected from the group of an RGD-containing peptide and an antibody that selectively binds an α_v-containing integrin.

72. The method of claim 69, wherein said molecule is CDCRGDCFC (SEQ ID NO: 14).

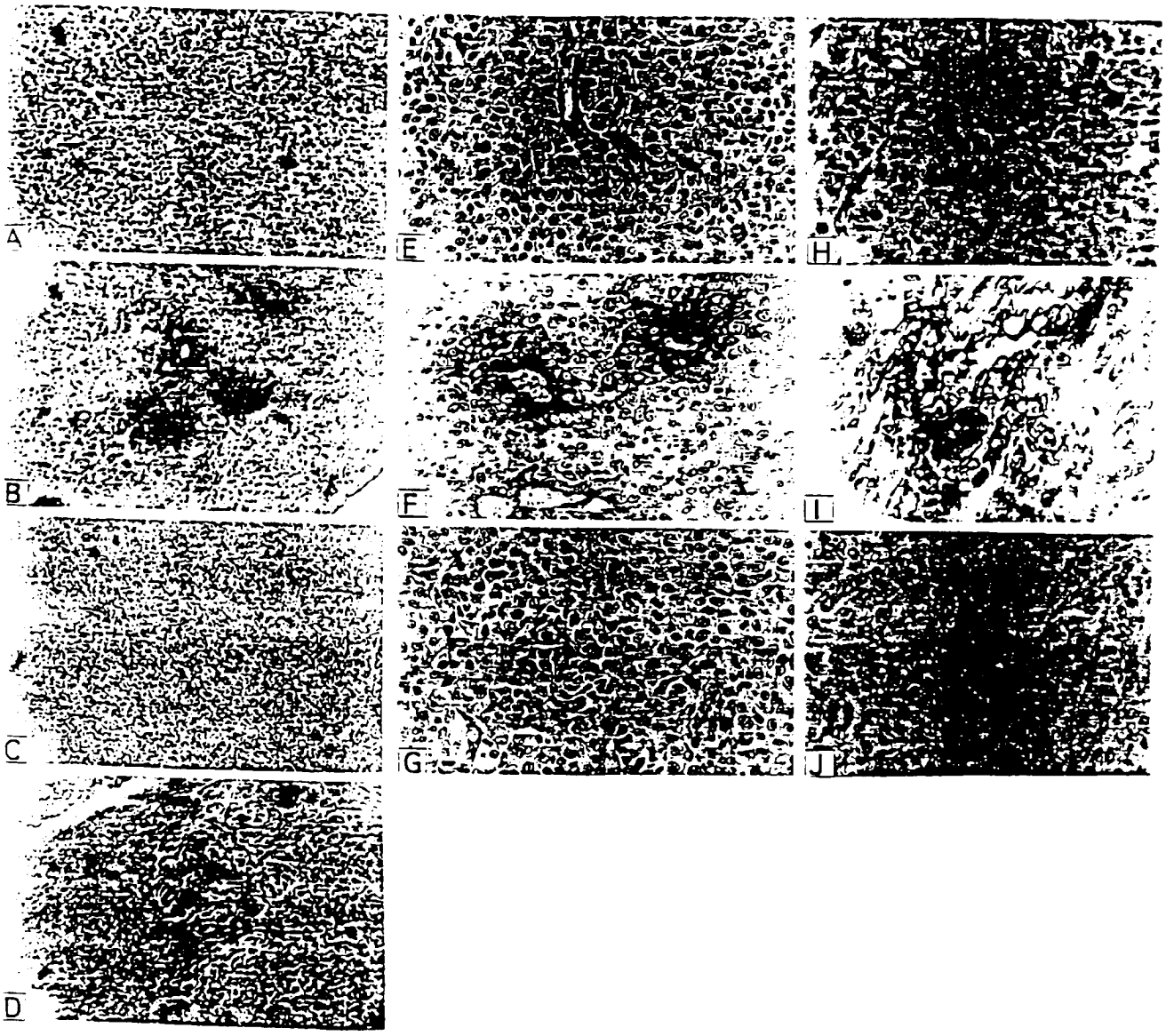

Figure 1

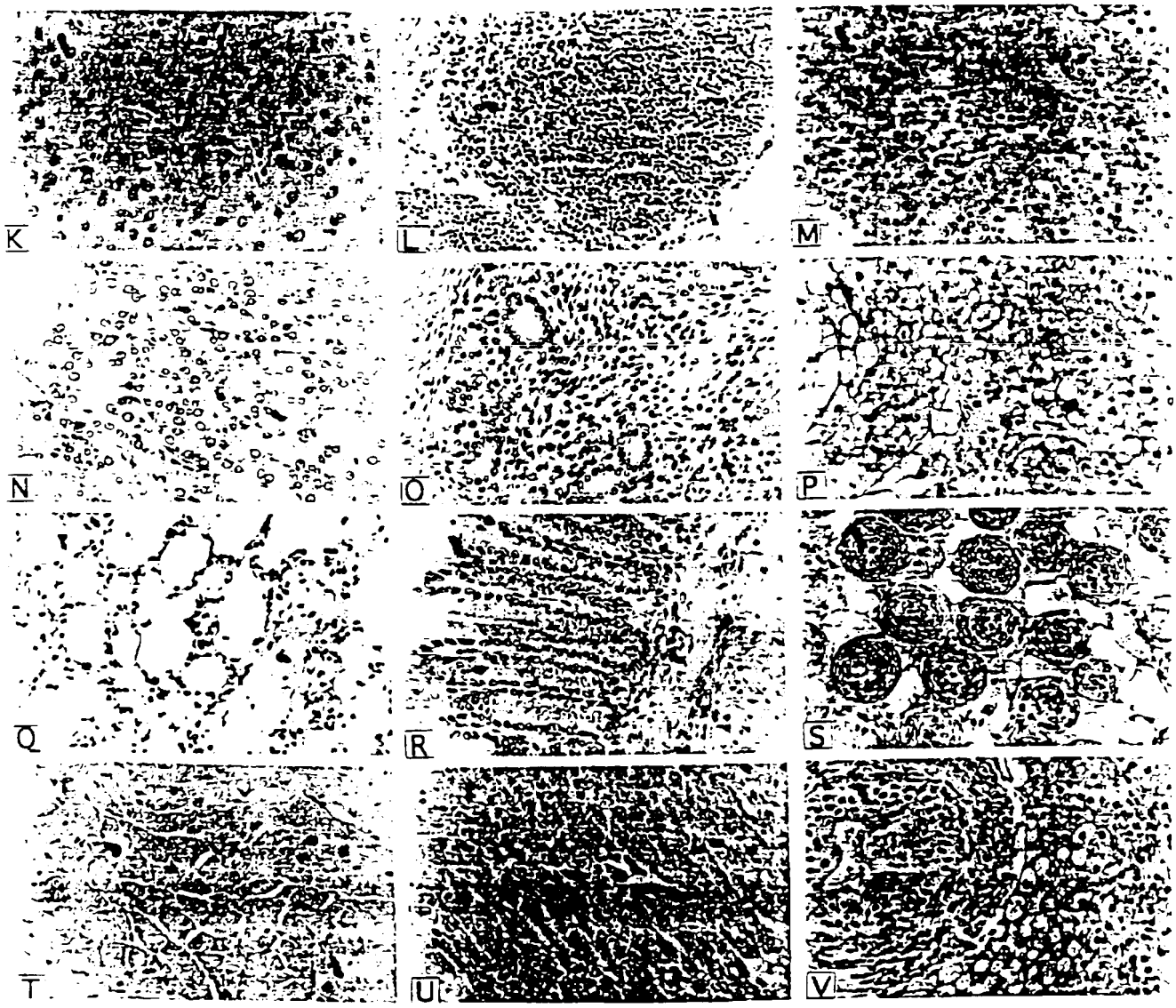

Figure 1 (cont.)

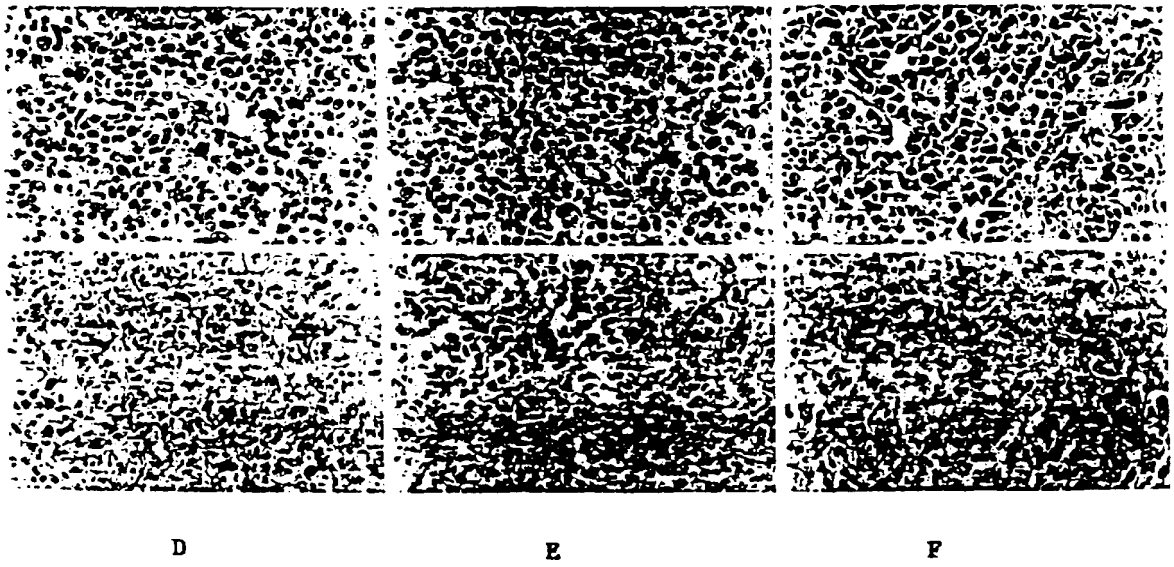

Figure 2